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Collagen Components in the Consecutive Extracts of Rat Skin

By

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Abstract

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The present results demonstrate that the various fractions of skin collagen are very different and that the solubility is a matter of definition. The methods of various authors for the preparation of the soluble fractions of collagen vary greatly. Standard procedures and a more defined terminology are urgently needed.

The purpose of this note is to draw attention to the quite different component patterns in the collagen fractions which have been obtained with various solvents.

That fraction of collagen which is soluble in dilute solutions of neutral salts is considered to be the youngest (Jackson 1957). It is assumed that collagen is continuously converted to less soluble and more mature forms by the formation of intra- and intermolecular cross-links, and that in growing connective tissue there is a continuous spectrum of various aggregates of different ages (Jackson 1960, Heikkinen *et al.* 1964a, b). By the present methods it is possible to test the properties of collagen solutions in the terms of the α-, β- and γ- (or x)-components (Näntö *et al.* 1964) and consequently of the intra- and intermolecular cross-links.

Various collagen fractions were obtained from skins of young rats (25—30 g) according to the flow sheet below where the amounts of each fraction are indicated. Only 11.3 per cent of total collagen remained in the final insoluble fraction. In Fig. 1. the starch-gel electrophoretic patterns of the components of denatured collagen fractions are presented. Collagen which is extractable with dilute salt solution consists mainly of non-linked α-components. When the ionic strength of the solvent is increased, additional collagen is dissolved and it contains also β-components with intramolecular cross-links. In acetic acid collagen swells and a great part of the residue dissolves. This fraction is characterized by a dominance of β-components and of larger aggregates, the x- (or γ-, δ-) components. Denaturation

TABLE I. Flow sheet of the extraction and purification of different collagen fractions from rat skin. If not stated otherwise, the manipulations were carried out at +4°C and the centrifugations with refrigerated equipment for 60 min at 35,000 × g

Rat skin
was homogenized in 0.15 M NaCl (2.5 × wet weight) with Sorvall-homogenizer for 2 min. After 24 hr the mixture was centrifuged and the extraction was repeated four times

Residue was extracted four times with 0.30 M NaCl and centrifuged		Combined supernatants were filtered and dialyzed against 0.1 M citrate buffer, pH 3.6. Collagen was precipitated with sodium chloride (final concn. 15%), collected by centrifugation and dissolved in 0.1 M acetic acid. This procedure was repeated twice. Collagen was finally precipitated by dialysis against several changes of 0.01 M disodium phosphate and water, collected by centrifugation, dissolved in 0.1 M acetic acid and lyophilized. The yield of 0.15-M NaCl-soluble fraction was 11.3% of total collagen
Residue was extracted four times with 0.45 M NaCl and centrifuged	Combined supernatants were handled as the 0.15-M supernatant. The yield of 0.30-M NaCl-soluble fraction was 21.4% of total collagen	
Residue was extracted four times with 0.5 M acetic acid and centrifuged	Combined supernatants were handled as the 0.15-M supernatant. The yield of 0.45-M NaCl-soluble fraction was 18.2% of total collagen	
Residue was extracted once with 0.5 M acetic acid at +40°C for 30 min and centrifuged		Combined supernatants were purified by precipitation with NaCl (final concn. 10%) and subsequently by dialysis against several changes of 0.01 M disodium phosphate and water. Collagen was collected by centrifugation, dissolved in 0.5 M acetic acid and lyophilized. The yield of acetic acid-soluble fraction was 29.4% of total collagen
Residue was lyophilized. The yield of the insoluble fraction was 11.3% of total collagen	Supernatant was lyophilized. The yield of warm-acetic acid-extractable fraction was 8.4% of total collagen	

at rather low temperature (at + 40° C for 30 min) yields a further soluble fraction containing proportionally more of these larger fragments (cf. Pikkarainen *et al.* 1964) with intermolecular bonds. The last insoluble residue may be solubilized to collagen components by digestion with pepsin. Other work (Lampiaho *et al.* 1965) shows that fractions of different metabolic ages can be demonstrated in conventional preparations of insoluble collagen.

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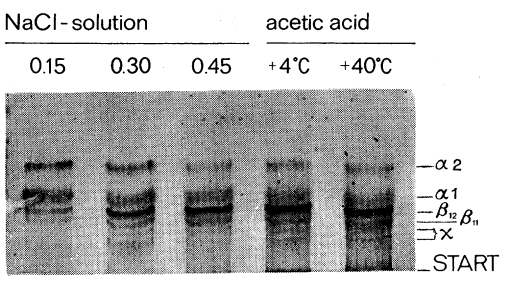

Fig. 1. Starch-gel electrophoretic patterns of the consecutive collagen fractions from rat skin. The preparations are described in the flow sheet.

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